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EDITORIAL

RINDERPEST IN THAILAND AND ITS LESSON

Diseases caused by germs have killed, maimed and scourged the world during all the time for which there is any record. No doubt the land was ravaged also in prehistoric times. All diseases of an infective origin, wax and wane in their incidence, virulence, and in the effects which they produce. Sometimes their virulence is so severe that whole communities can be struck down and paralysed in a lightning attack. Other invasions are of a milder order and have little or no immediate lethal effect but continue to harass the victims as would a mouldering sore. The property of virulence does not depend entirely upon the invading organism. The victims, whether they be of the animal or vegetable kingdom, are endowed with a means of defence and, if this be of high standard, the attackers can be destroyed or weakened to such an extent that all their malignant efforts fail. This natural defence mechanism waxes and wanes also. It is prompted and stimulated by attack. As a rule when the invaders are persistant in their efforts the resistance, amongst those that survive the first attack, reaches a very high level. On the other hand if the assault be weak or if it be postponed for a period, the defensive processes slumber, become lethargic and lose their resilience. At such a time the victim is in great peril and at the mercy of any germ, even those of low lethal potential.

In controlling epidemics of germ born disease one can follow two lines of action. One can try to eliminate the offensive germs. There are instances in which this method has been followed with some success. The best examples are manifested in the campaigns against malaria and tuberculosis. In the first instance by destroying the host vector, the anophelene mosquito, the life cycle of the malarial parasite is interrupted and the species dies, in the second instance the process of elimination is a direct one against the invading organism the tubercule bacillus. There can be no doubt that these two campaigns have given good results but an elimination method can only be a complete and lasting success if all the harmful organisms are destroyed. This is a difficult end to achieve. If it cannot be accomplished there may be grave peril in the future; in a few generations natural resistance, because of the absence of

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stimulation, may fall to such a low level that the few organisms which survive may be able to light a fire which soon could get beyond control. The second line of action is to make use of "metabolic warfare" and imitate Nature's own method of defence by stimulating the forces of resistance. It has the advantage that the offensive organisms can be ignored, for if the bodily defences are enhanced to a sufficiently high degree the enemy attacks in vain. This is the ideal method to use in those instances when diseases, commonly called plagues, spread in epidemic fashion through the animal and human populations. The word plague has acquired an ominous sound and meaning for it betokens the widespread dissemination of pestiferous disease. The world has suffered many plagues some of great and others of lesser degree when assessed by the amount of suffering and economic loss that has been occasioned.

The Black plague of man (Bubonic plague) and Cattle plague (Rinderpest) come to mind immediately for there must be few areas in the world that have not suffered from their depredations. Both plagues have been frequent and constant visitors. The story of the Great Plague of London in the 15th Century can still chill a reader's heart and the havoc wrought by Rinderpest in Africa and the East, and in this country in the 19th Century, cannot be ignored. Rinderpest can lead to great suffering and disaster by famine and economic ruin. Fortunately, owing to the pioneer work in India of the late Dr. J. T. Edwards, a method of protection and control was evolved. By passaging the virus through goats its lethal properties were attenuated and a protective vaccine could be prepared. Further work has shown that sometimes the humble rabbit may stand in the goat's stead.

Eight years ago Thailand, a country which had been ravaged again and again by Rinderpest, was cleared of the fell disease. In February 1958 following outbreaks in neighbouring countries it made its appearance again and by the end of that month 120 outbreaks had been identified. F.A.O. sent J. R. Hudson to Thailand to help and advise. The veterinary services were mobilised quickly and by drastic police measures, including slaughter and vaccination, the epidemic was brought under control. In 1959 Thailand is free from Rinderpest again. The trouble in Thailand may do the world good. It emphasises the fact that in the control of epidemic disease there can be no respite; whatever the method of control, elimination or the stimulation of resistance. Under present circumstances one cannot hope to eliminate the species of any invading organism and safety depends upon vigilance, the constant application of sanitary measures and protective vaccination.

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THE EFFECT OF FEEDING HAY OR SILAGE ON THE INCIDENCE OF KETOSIS IN DAIRY COWS

By

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In previous work (Blackburn, Castle & Strachan, 1958) it was shown that the feeding of different weights of concentrates in the 6 weeks before calving had no effect on the subsequent incidence of ketosis in two groups of dairy cows. The rations of both groups had included grass silage and the possibility of some connection between the feeding of silage and the incidence of ketosis had been considered.

A relationship between the consumption of silage, and the level of acetone in the blood of cattle and in the number of animals excreting ketones in the milk has been shown by a number of workers (Brouwer & Dijkstra, 1938; Potts & Kesler, 1957; Penny, 1958). Reasons for an apparent association between silage feeding and ketosis have been discussed by Johnson (1951), and Adler, Roberts & Dye (1958) have reported clinical observations which indicate that on a herd basis silage may be a significant predisposing factor in outbreaks of ketosis.

This paper reports a trial in which the effects of rations containing either silage or hay on the incidence and severity of ketosis were investigated.

Cow number	*Liveweight (lb.) 3 weeks before calving*	*Age January 1958 (years)*	*Date of calving 1958*
Hay and fodder-beet			
1	1040	3½	4 Feb.
2	1340	6½	12 Feb.
3	1305	7½	20 Feb.
4	1100	7*	9 Mar.
5	1150	5	10 Mar.
6	1200	5½	16 Mar.
7	1390	8½	13 Mar.
Mean	1220	6½	
Silage and dried grass			
8	1105	3½	24 Feb.
9	1430	8*	15 Feb.
10	1300	8	2 Mar.
11	1205	7	1 Mar.
12	1120	4½	7 Mar.
13	1220	5½	16 Mar.
14	1470	8½	22 Mar.
Mean	1260	6½	

* Approximate

TABLE 1

Details of the cows in the two groups, one of which received a basal ration of hay and fodder-beet, and the other a ration of silage and dried grass.

Experimental

Cows

Fourteen Ayrshire cows which calved between 4th February and 22nd March were used. Six of these animals had been on the trials one year previously (Blackburn *et al.*, 1958). In December the cows were paired so that members of each pair were as similar as possible in liveweight, age and date of calving. Details are given in Table 1. The animals in each pair were allocated at random to one or other of the two experimental treatments. They had all been similarly fed and managed up to this time.

Feeding

From 27th December, i.e. 39 days before the first cow calved, the cows were fed one of the two following basal rations according to their group:—

Hay group (lb./day)		
Hay	12	(10 lb. after 28th January)
Fodder-beet	56	
Silage group		
Grass silage	60	
Dried grass	9	

Apart from the reduction in the amount of hay after 28th January these rations were fed throughout the experimental period until 16th April when the trial ended.

Before calving all the 14 cows were fed a 'steaming-up' ration of concentrates at the following rate:—

	Weight of concentrates (lb./cow/day)						
Weeks before calving	6	5	4	3	2	1	Total in 6 weeks
	2	3	4	4	6	8 =	189

This level of feeding was a mean of the two levels fed in the earlier experiment (Blackburn *et al.*, 1958).

The composition of the concentrate mixture and of the other foods fed during the experiment is given in Table 2. On the day after calving and for the following 14 days the hay group were fed 12 lb. concentrates per head per day and the silage group 8 lb.; this difference resulted from the higher nutrient content of the silage and dried grass ration. From the 15th day onwards the hay group were fed concentrates at the rate of 4 lb. for every 10 lb. of milk produced. The silage group also were fed from the 15th day at the same rate but only for the milk produced after the first 10 lb. since the silage ration supplied sufficient nutrients for maintenance and the production of 10 lb. milk a day. The amount of concentrates to be fed was adjusted once per week and the ration for each cow was weighed individually every day.

All the foods in the two basal rations had been grown on the farm. The hay, silage and dried grass were all produced from leys containing little or no clover and which had received heavy dressings of balanced artificial fertilizers. The hay was cut at the early flower stage and was well made, green and leafy. The silage to which no preservative had been added had been cut from grass at the long leafy stage. The

dried grass was cut at a "leafier" stage than the silage and came from a number of different fields. The hay, fodder-beet, silage and dried grass were weighed in bulk daily throughout the experimental period and then divided carefully between the seven cows in each group.

Concentrate mixture			
	% by weight	*Composition as fed (%)*	
Oats (bruised)	40	Dry matter	84.4
Barley (ground)	20	Crude protein (by analysis)	14.4
Flaked maize	10		
Soya meal	10	Approx. starch equivalent	66
Locust bean meal	5	Approx. digestible crude protein	11.0
Tic beans	5		
Groundnut cake	5	CaO	0.19
Grass meal (ground)	5	P_2O_5	0.75

Other foods				
	Hay	*Fodder-beet*	*Silage*	*Dried grass*
Dry matter (%)	84.0	19.2	19.2	87.2
Percentage of dry matter				
Crude protein	8.7	8.6	11.2	11.7
Approx. starch equivalent	36	50	50	56
Approx. digestible crude protein	5	4	7	7
CaO	0.63	0.15	0.94	0.66
P_2O_5	0.45	0.33	0.68	0.62

TABLE 2

The composition and calculated feeding values of the concentrate mixture and of the other foods used in the basal rations.

The hay group was fed hay after the evening milking and fodder-beet at 7.00 a.m. after the morning milking, and again at 12 noon; the silage group received dried grass in the evening and silage at the same times as the other group received fodder-beet.

Samples of the concentrates and the fodder-beet were taken twice a week, the hay and dried grass once per week from a daily bulked sample and the silage once per day. The dry matter content of each sample was determined and crude protein analyses done on weekly bulked samples. Detailed analyses of the organic acid content of the fresh silage and of the CaO and P_2O_5 contents of all the foods were made.

Housing, weighing and milking

The cows were housed during the experiment in a double-stall byre but were allowed into a bare paddock each day from 10.30 a.m. to 12 noon. They were weighed on one morning each week at approximately 12 noon. Both the cow and the calf were weighed within 12 hours of the cow calving. The calf was then separated from the cow which thereafter was milked twice a day.

Blood samples and analysis

A sample of blood was taken from each cow once per week for a minimum of 6 weeks before calving, and for 6 to 9 weeks after calving. The total acetone bodies in each sample were determined by the method of Thin & Robertson (1952).

The circulating eosinophil content of the blood was measured since this has been reported to be one of the factors affected during ketosis. Carlström (1950) observed that with ketosis there was an increase of 15-40% in the eosinophil leucocyte content of the blood, and Shaw (1956) has reviewed at length this relationship between the blood eosinophils and ketosis.

The blood was stained by a modification of the method described by Randolph (1949). The stock solution used for staining consisted of 0.1% phloxine in 50% propylene glycol diluted with an equal volume of distilled water. With this solution a period of 10 min. was required for maximum staining and the eosinophil cell counts were then made using the standard pipette and counting chamber.

Milk tests

From the day after calving until the end of the experiment a sample of milk from each cow was subjected daily to a controlled Rothera's test and, according to the colour reaction obtained, it was graded negative (−), doubtful (±), or +1, +2, +3, +4 or +5.

Treatment of cows

When the milk of any of the cows gave a test of +3 or higher she was treated at once in an endeavour to avoid a more severe clinical condition and a loss of milk.

In view of the encouraging reports in the literature of the value of prednisolone in the treatment of bovine ketosis (Link, Newton & Huber, 1957; Paterson, 1957; Huber, 1958) it was decided to investigate the value of this substance. During the course of the trial it was decided to treat animals on 24 separate occasions. Twelve of these treatments consisted of an intramuscular injection of 100 mg. of crystalline prednisolone* whilst the other twelve were of a more conventional type. These other treatments were one or more doses of 400 ml. of glucose solution (50%) intravenously, 4-6 oz. of sodium propionate as a drench for three consecutive days, mineral supplements added to the concentrates, drenching with glycerine, and the addition of potassium permanganate to the drinking water.

The prednisolone and the other comparative treatments were given alternately as required throughout the trial. The response to a treatment was measured by changes in milk yield and in the ketone bodies in the blood when next sampled.

Dates

The experiment lasted from 27th December 1957 to 16th April 1958.

Results

Food consumption

There were very few refusals of food throughout the trial either when the cows were being 'steamed up' or after calving when the animals were in a normal state of health, but with the onset of clinical symptoms of ketosis food refusals did occur. When this happened and the animal was treated, every effort was made to ensure that she ate her experimental diet as soon as possible after treatment.

Excluding the periods of food refusals, the mean daily consumption of dry matter in the basal ration of the hay group was 19.2 lb. containing 8.4 lb. of S.E. and 0.83 lb. of D.C.P. Using the standard of Woodman (1957) plus the 10% addition suggested

*'*Deltatcortril*' brand of prednisolone: Pfizer & Co., Inc.*

by Blaxter (1956) this ration was more than adequate and had, in fact, a surplus of 1.8 lb. S.E. above normal feed requirements. The basal diet of the silage group contained 19.4 lb. of dry matter which included 10.2 lb. of S.E. and 1.36 lb. of D.C.P., sufficient nutrients for maintenance and the production of 10 lb. of milk with even then a surplus of 0.8 lb. of S.E. Of the concentrate mixture 4 lb. were fed for each 10 lb. of milk produced except for the first 10 lb. on the silage ration. This amount supplied 2.6 lb. of S.E. and 0.44 lb. of D.C.P. In total, therefore, both the experimental rations supplied similar amounts of nutrients and there was always an adequate amount of S.E. although the intake of protein was lower than the normal recommended standards (Woodman, 1957).

The CaO and P_2O_5 content of the foods are given in Table 2. From these and the known intakes of dry matter from the various foods the consumption of these minerals was calculated. Cows yielding 40 lb. of milk per day in the hay group had a daily intake of 1.6 oz. CaO and 3.1 oz. P_2O_5, whereas in the silage group the intakes were 2.9 oz. and 3.5 oz. respectively.

Silage quality

The grass silage was of only medium protein quality (Table 2) but had all the characteristics of a well-fermented acid silage. It had a keen pleasant smell and a pH of 4.02. The lactic acid content of the silage dry matter was 4.20%. The percentage of steam-volatile fatty acids (also on a dry matter basis) were as follows: formic, 0.04; acetic, 1.71; propionic, 0.25; iso-butyric, 0.27; *n*-butyric, 0.91; higher, 0.27. The ratio of the weight of lactic acid/100 g. dry matter to the sum of the weights of acetic, *n*-butyric and iso-butyric acids/100g. dry matter was 1.5 : 1.0 which, coupled with the low pH, indicates a satisfactory acidification (Watson & Smith, 1951).

Incidence of ketosis and blood values

The values for the content of ketone bodies in the blood are given in Table 3. It will be seen that even before calving the level of ketone bodies was high. In the hay group the mean value for the seven cows on the six tests was 12.0 mg. with a maximum value of 24.7 mg. for cow 5 on one occasion. Six of the seven cows had values of over 10 mg. and, on the grouping of Thin & Robertson (1953), were already sub-clinical cases. In the silage group the mean value before calving was 7.5 mg. and four of the animals could be classed as sub-clinical. In both groups there was a tendency for the ketone values to decline somewhat nearer the calving date but throughout this pre-partum period the hay group was consistently higher than the silage group.

After calving the ketone values increased in a number of the cows, but, as stated earlier, treatment was started at an early stage to avoid, if possible, the severest symptoms. As an indication of the degree of ketosis in the cows in the two groups they have been sub-divided arbitrarily into groups based on the level of the total ketones in the blood according to the method of Thin & Robertson (1953), which is as follows.

		mg. acetone/100 ml. blood	
Group	A	>60	severe clinical cases
„	B	59—30	milder „ „
„	C	29—10	sub-clinical „
„	D	<10	normal

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Cow number	Days before calving 42-36	35-29	28-22	21-15	14-8	7-0	0-7	Days after calving 8-14	15-21	22-28	29.-35	36-42	43-49	50-57	58-63
Hay and fodder-beet															
1	—	13.0	11.9	13.3	11.5	15.3	26.8	53.9	34.1	13.8	30.5	22.3	11.5	8.6	18.0
2	4.9	7.1	9.3	5.2	4.9	7.3	21.0	3.2	5.2	14.4	5.1	4.4	4.1	1.1	5.7*
3	7.1	8.6	6.8	5.6	11.0	11.2	3.4	21.8	28.5	23.7	12.8	28.9	5.1	13.3*	4.1
4	19.9	14.0	16.4	13.8	10.6	6.2	5.9	18.2	35.7	14.9	38.2	32.5*	2.8	18.8	
5	21.7	19.0	17.0	24.7	10.7	17.6	8.0	13.7	4.9	6.1	8.8	16.9*	34.9	37.4	
6	16.4	15.0	14.5	4.1	4.1	9.3	5.7	3.2	3.7	5.2	5.7*	2.8	4.5		
7	9.6	12.5	14.8	19.0	10.6	14.4	19.5	28.4	24.7	29.7	42.6*	30.1	10.1		
Mean	13.3	12.7	13.0	12.2	9.1	11.6	12.9	20.3	19.5	15.4	20.5				
Silage and dried grass															
8	16.4	7.3	6.8	15.9	7.6	9.6	6.0	7.8	43.9	28.4	10.7	4.8	12.0	16.9*	10.1
9	5.7	4.1	4.1	6.8	7.2	7.1	11.0	12.2	13.3	13.7	6.2	3.2	5.9	6.7	6.3*
10	10.1	6.6	4.6	4.5	5.8	2.3	10.6	16.1	11.5	7.6	8.8	4.6	8.3*	22.7	4.1
11	8.3	8.8	13.3	8.1	8.8	5.2	13.0	14.4	26.8	18.5	24.4	33.3	7.8*	3.6	5.2
12	6.2	7.6	6.3	5.8	6.0	2.9	9.3	6.7	25.2	7.8	29.3	8.6*	12.3	2.3	
13	12.0	5.9	9.3	5.8	7.8	6.1	7.6	7.6	7.1	18.0	8.6*	12.3	14.5		
14	5.6	14.5	6.7	4.6	6.1	9.3	5.4	9.6	22.4	8.3*	21.8	6.7			
Mean	9.2	7.8	7.3	7.4	7.0	6.1	9.0	10.6	21.5	14.6	15.7				

* Cows out to grass after this time.

TABLE 3

Content of ketone bodies in the blood of the cows in the two groups (mg. acetone/100 ml.)

The results of this grouping for the 6-week period after calving are given in Table 4. Blood values determined after 6 weeks have not been used in this grouping since after this time a change had been made in the rations of a number of the cows. From Table 4 it will be seen that the incidence and severity of the ketosis was very similar in the two groups with a tendency for the hay group to have the larger number of higher values. According to the blood values there were no severe clinical cases.

Rothera's test

The number of days on which samples of milk were positive to Rothera's test and the grading according to the intensity of the colour reaction are presented in Table 5. It will be seen that the total number of doubtful and positive tests was almost the same in both groups and that there was little variation between them in the distribution of the colour grading. In the hay group 69 of the 111 tests (62%) were graded +1 or +2 whereas in the silage group the figure was 66 out of 107 (also 62%). Thus, judged on the basis of Rothera's tests it was clear that there was little or no difference between the two experimental rations in their effect on the acetone bodies in the milk.

Eosinophil counts in the blood

In both groups of cows there was a marked decrease in the number of eosinophils per cub. mm. of blood from the period 29-35 days before calving until the time of calving. This decrease continued for a further 7 days after calving in the hay group and for 14 days in the silage group after which there was a small increase in both groups up to 21 days after calving. Thereafter each animal had a somewhat different individual pattern but the patterns were all characterized by a tendency to an increase in eosinophils with increasing signs of clinical ketosis and a marked and sudden reduction in numbers after injections of 100 mg. of crystalline prednisolone. Because of these injections which were made at an early stage in the development of the ketosis it was not possible to make an accurate comparison between the blood picture of the two groups but in general there was little difference between them.

	Group			
	A	*B*	*C*	*D*
	Ketone bodies (mg. acetone per 100 ml.)			
	>60	59-30	29-10	<10
Ration	*No. of cows*			
Hay and fodder-beet	Nil	3	3	1
Silage and dried grass	Nil	2	5	Nil

TABLE 4

The number of cows grouped according to the maximum value of ketone bodies in the blood during the 6-week period after calving.

Milk yield

The mean daily milk yield per cow over the first 56 days of the lactation was 43.3 lb. for the hay group and 43.5 lb. for the silage group. The clinical and the sub-clinical ketosis reduced milk production, and since its incidence was about the same in both groups, it can be inferred that the reductions in milk yield were about the same in both experimental groups. As in the previous trial (Blackburn *et al.*, 1958) milk production fell as the level of blood ketones increased, and, instead of normal

lactation curves with a well defined peak a few weeks after calving, the curves were very irregular with a number of more pronounced peaks and troughs. These irregular and abnormal lactation curves were recorded for each animal in both treatments.

Liveweights

The liveweights of the cows and the mean values for each group are given in Table 1. It will be seen that the silage group was, on average, 40 lb. heavier than the hay group, and this difference was maintained with fluctuations throughout the experiment. The mean difference between the two groups over the entire period of the trial was in fact 46 lb. in favour of the silage group. After calving the hay group lost an average of 126 lb. in 42 days compared with 120 lb. for the silage group in the same period. The mean birthweight of the calves on the hay and on the silage treatments were 69 and 72 lb. respectively.

Clinical symptoms

A clinical picture of the animals in the two groups based on daily observations of food refusals, fluctuations in milk yield, alertness, condition of the faeces and of the appearance of the animal showed no major difference between the feeding treatments. One animal in each group was classed as normal (Nos. 6 and 9) and two were classed as sub-clinical (Nos. 2, 3, 10 and 13). In the hay group two animals were graded as mild clinical (Nos. 1 and 4) and two as clinical (Nos. 7 and 5), whereas in the silage group three were graded as mild clinical (Nos. 8, 11 and 12) and one as clinical (No. 14). These observations were made before the analyses of the blood samples were completed, but none the less the agreement between them and the results in Table 4 for the ketone bodies in the blood was quite close. In eleven of the cows these two methods of classifying the cows agreed very well although with the other three animals the blood values indicated a more severe ketosis than the clinical symptoms suggested.

	Reaction of milk						Total
	±	+1	+2	+3	+4	+5	
Ration							
Hay and fodder-beet	14	43	26	26	2	0	111
Silage and dried grass	10	39	27	21	10	0	107

TABLE 5

The total number of days on which samples of evening milk from the two groups of cows were positive to Rothera's test.

Treatment results

Of the ten animals which received a single dose of prednisolone only one animal showed a marked and permanent increase in milk yield. Eight of the cows all gave a temporary rise in yield but this quickly fell and in one animal the milk yield continued to decline. One animal was given two doses at an interval of 15 days and recovered her normal milk yield. The effect of the prednisolone on the ketone content of the blood was very erratic and in only three of the eleven animals was there a marked downward trend.

The results of the other comparative treatments were equally negative, and any small increases in milk yield were of a temporary nature and soon relapsed.

Until the end of the experiment when the cows went out to pasture no treatment had any lasting effect on reducing the ketosis. As in the previous trial (Blackburn *et al.*, 1958) spring grass had a marked anti-ketogenic effect although this effect was slow on cows with the severest clinical symptoms.

It is concluded that under the conditions of this particular trial, the prednisolone was as ineffective a treatment for bovine ketosis as were the more conventional treatments.

Other metabolic diseases

Two cows in the hay group and three in the silage group had milk fever at the time of calving. All were treated successfully with calcium borogluconate.

Discussion

This experiment was conducted in a herd in which ketosis had been a major problem for at least the three previous winters. Grass silage had always been fed to the dairy herd during the winter months and, as stated earlier, this trial was made because of suggestions in the literature that there may be a connection between silage feeding and ketosis.

The two basal diets which were compared were of a contrasting nature. The one containing hay and roots was a ration which supplied maintenance requirements only and could be classed as an average farm ration. The type of roots used are not very widely grown but are merely equivalent to a larger amount of mangolds (Bailey, Castle & Foot, 1953). The silage and dried grass ration covered maintenance requirements and the production of a gallon of milk, and is the type of ration which is fed on farms where the standard of grassland husbandry is higher than average. The weight of silage fed, 60 lb./day, contributed approximately one-third of the animals' total daily intake of dry matter and by normal feeding standards would be classed as a fairly large amount of silage. In the previous year only 28 lb. silage per day had been fed to each animal and it was hoped that by more than doubling this quantity useful information would be gained about its relationship with the ketosis.

From the results of the trial it is quite clear, however, that the cattle on both the experimental basal rations had a very similar incidence of ketosis. This was seen from the level of ketone bodies in the blood, the results of Rothera's tests and the clinical picture of the affected animals. If, therefore, silage feeding was a predisposing factor in the outbreaks of ketosis in the silage group then obviously the hay and root ration was equally suspect. The silage had shown a satisfactory fermentation during its formation and unlike that described by Adler *et al.* (1958) it was not of the butyric type. The silage *per se* could not be directly incriminated as a cause of ketosis and in our present experiment there was obviously some other reason. Both rations supplied an adequate amount of dry matter containing sufficient starch equivalent for maintenance and production even when the extra 10% suggested by Blaxter (1956) was added to the requirement. Fodder-beet is a particularly good source of readily available carbohydrates (Bailey, Castle & Foot, 1953). The intake of protein was slightly lower than the recommended standard (Woodman, 1957) but other trials in this herd had

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shown that a concentrate with a protein content as low as 10.4% D.C.P. was as effective for milk production as one with a higher protein content (Holmes, Waite, MacLusky & Watson, 1956). Because of this a low protein concentrate had been used in the present trial.

The daily intakes of CaO and P_2O_5 were below the recommended amounts suggested by Woodman (1957) on the hay and root ration. On the silage ration the intakes of both CaO and P_2O_5 were adequate. However, in view of the lack of precise knowledge on the requirements of these two minerals for dairy cattle (Woodman, 1957; Duncan, 1958) it is unrealistic to implicate these minerals until more trials have been conducted.

Grass silage is a relatively cheap source of nutrients for the dairy cow (Hamilton, 1955; Moore, 1958) and is also a product which fits into a system of intensive grassland management extremely well. When carefully made it is a useful food which can replace expensive purchased concentrates. From the results of the above trial there would appear to be no direct connection between the feeding of silage and the incidence of ketosis, and until more experimental work is done there is every reason for continuing to make *good* grass silage and to feed it to dairy cattle.

Summary

1. Fourteen Ayrshire cows aged from $3\frac{1}{4}$ to $8\frac{3}{4}$ years were divided into two balanced groups based on age, liveweight and date of calving.

2. One group of cows was fed 10-12 lb. of hay and 56 lb. of fodder-beet per day, whereas the other group was fed 60 lb. of grass silage and 9 lb. of dried grass per day. These rations were fed before and after calving.

3. Before calving both groups of cows were fed an equal amount of concentrates as a steaming-up ration. After calving the hay group was fed 4 lb. of concentrates per 10 lb. of milk and the silage group at the same rate but only after the first 10 lb. of milk.

4. In both groups after calving there were six cases of ketosis. The hay group had two sub-clinical, two mild clinical and two clinical. The silage group had two sub-clinical, three mild clinical and one clinical.

5. The mean levels of ketone bodies in the blood expressed as mg. acetone per 100 ml. were 12.0 and 7.5 in the hay and silage groups respectively before calving. After calving the values increased and three cows in the hay group and two in the silage group reached values in the 30 to 59 mg. range.

6. In the hay group 111 samples of milk were positive to Rothera's test compared with 107 in the silage group, and in each of the two groups 62% of the samples were graded +1 or +2 in Rothera's test.

7. The average milk yields, the changes in liveweight of the animals, and the changes in the eosinophil count in the blood were all very similar for both groups.

8. It is concluded that the two contrasting types of basal rations did not differ in their effect on the incidence and severity of ketosis.

The authors thank Mr. J. N. Watson for assistance with the cows, Dr. D. G. Armstrong for the analysis of the silage, and Mr. R. C. Voss of the West of Scotland Agricultural College for the mineral analyses of the foods.

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LEIOMYOMA OF THE INTESTINAL WALL IN A DOG

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Introduction

Tumours of smooth muscle are not very common in the dog. Willis (1953) found 6 leiomyomata among 204 canine tumours, but did not mention the sites from which they were removed. The vagina is probably affected most frequently; thus, Cotchin (1954 *a*) found 3 leiomyomata in a series of 27 vaginal tumours. During a period of about 4 years the Clinical Pathology Department here at Langford recorded 3 cases of vaginal leiomyoma, a single uterine lesion and a case of multiple leiomyomata in the perineal region of a bitch. The incidence in the male dog is even less than in the female, though Smith and Jones (1957) recorded a leiomyoma in the region of the prostate gland, at which site such tumours occur occasionally in man (Patch and Rhea, 1935). Cotchin (1954 *b*) has observed a leiomyosarcoma in the urinary bladder of a bitch. Reliable reports of leiomyomata in the alimentary tract of the dog are very rare indeed, though such neoplasms are of fairly frequent occurrence in human beings and were classified as " inner " (submucosal) or " outer " (subserosal) by Steiner (1898). Paukul (1907) described a leiomyoma in the intestine of a greyhound. Among 160 alimentary tract tumours, in a series of 2,361 miscellaneous canine neoplasms, Cotchin (1954 *a, b, c*) recorded a single leiomyoma of the stomach and another which occurred in the anal region.

The following report describes an apparently benign smooth muscle tumour which was removed by surgery from the abdomen of an Alsatian bitch.

Case History

The subject, a 7 years old pedigree Alsatian bitch, was presented to a Veterinary Surgeon for treatment of severe bilateral *otitis externa* associated with increasing lassitude and depression. In the course of general examination the Veterinary Surgeon palpated a large firm mass within the abdomen.

The aural inflammation was treated successfully, and the bitch was referred to the Department of Veterinary Surgery, University of Bristol, for further investigation of the abdominal mass.

Although its temperature and respiration were normal at the time of admission, the animal was lethargic and had a poor appetite. The abdomen was distended and pendulous. There was no history of vomiting or diarrhoea, nor did either appear prior to removal of the tumour.

On palpation of the abdomen a " heavy " firm swelling was found, the contours of which appeared definite but rounded. Its size prevented wide manipulation but the mass appeared to be unattached to the abdominal wall. Palpation did not elicit pain or even marked discomfort, but manipulation of the mass was facilitated greatly by relaxation of the abdominal wall under general anaesthesia.

A diagnosis of neoplasia was made. Under anaesthesia induced by intravenous administration of " Intraval Sodium "* and maintained by nitrous oxide and oxygen, the abdominal cavity was explored via a midline incision.

Much of the cavity was found to be occupied by a large pinkish-white tumour-like mass. The surface was covered by peritoneum and the mesentery was not involved. When the mass was lifted from the abdomen it was seen to arise from the ileum. A careful inspection of the abdominal organs failed to reveal any signs of metastatic spread.

Because the tumour appeared to be confined to a small part of the alimentary tract and differed macroscopically from a lymphosarcoma there appeared to be a reasonable possibility that it was not highly malignant. It was excised, together with about 4 inches of bowel, and an end to end anastomosis was made. No untoward reactions were observed in the immediate post-operative period.

Penicillin therapy was maintained for 4 days, and a fluid diet was given whilst the bitch remained in the kennels. On the 7th day after the operation the skin sutures were removed and the animal returned home. The owners were advised to supply a semi-solid diet for a further 14 days. Thereafter recovery was uneventful.

When seen again, 258 days after extirpation of the tumour, the bitch appeared to be in fairly good general condition (Fig. 4) but was perhaps somewhat too fat. Nevertheless, she was bright, active and had an excellent appetite. Palpation of the abdomen failed to reveal any recurrence of the tumour, though the abdominal wall remained slightly pendulous.

Pathology

(*a*) *Macroscopic*

The tumour (Fig. 1), which was lobulated and nodular in appearance, measured 7.5 ins. x 4.5 ins. x 3.8 ins. and weighed 976 grammes; its greatest axis formed an acute angle with that of the intestine.

The neoplastic mass appeared to originate in the submucous connective tissues and, although a hemispherical protrusion partly occluded the lumen of the viscus, the intestinal mucosa was not ulcerated and remained intact. The principal direction of proliferation was towards the peritoneal cavity and the outer surface of the tumour was covered by peritoneum. On incision the tumour was found to be pinkish-white in colour and of a fairly firm consistency, though it resisted the knife less than most fibromata of similar size. At its origin the neoplastic tissue merged imperceptibly with the normal bowel wall. The size, structure and distribution of the tumour indicated that it was an " outer " or subserous leiomyoma.

(*b*) *Histological*

Histological sections showed that the tumour consisted primarily of interlacing bundles of narrow pointed cells with long rod-shaped nuclei whose extremities were

* Thiopentone sodium — May & Baker Ltd., Dagenham, Essex.

rounded (Fig. 2). A few areas showed a considerable admixture of fibroblastic collagenous elements among the smooth muscle cells, but in most fields the collagenous stroma was both fine and scanty. No true fibrous capsule could be found and, in the peripheral zones, bundles of neoplastic cells were interwoven with the normal smooth muscle of the intestinal wall.

The tumour mass was not highly vascular; but blood vessels of varying degrees of development were present and these were particularly elaborate and numerous near the more collagenous zones of the tumour. Where large vessels occurred in zones predominantly composed of muscle cells, the smooth muscle in the walls of the vessels appeared indistinguishable from that of the tumour, and cells from both sources often interdigitated.

Masson's trichrome stain emphasized the cytoplasmic nature of the tumour and the relative paucity of collagenous fibres; myofibrils in the cytoplasm were easily observed in sections stained by this method and in others stained by phosphotungstic acid haematoxylin. Gomori's technique demonstrated much reticulin, but that of Verhoeff showed that elastin fibres were relatively few in number. Cellular pleomorphism was not well-marked, though a few giant hyperchromatic nuclei were evident. Mitotic figures were very rare. The majority of nuclei were fat or rod-shaped with rounded ends (Fig. 3); cells with fusiform nuclei, considered to be indicative of rapid growth, were comparatively uncommon. Palisading of nuclei could be seen in a few fields, but this was not an obvious feature of the tumour.

Discussion

In the dog, pyloric carcinomata are the most common tumours of the stomach and lymphosarcomata are the most common tumours of the intestine; tumours of the latter type have the highest incidence of all alimentary neoplasms other than squamous cell carcinomata of the tonsil.

By comparison, the rarity of muscle cell tumours of any organ renders their occasional occurrence of considerable interest; and this low incidence of myomata among animals in general, and among dogs in particular, differs remarkably from their relatively high incidence in human beings. For example, Willis (1953) suggested that uterine myomata may occur in about 20 per cent. of women over 30 years of age and, according to Evans (1956), leiomyomatous neoplasms arising in the muscularis of the alimentary canal are the commonest form of primary mesodermal tumour developing in the human stomach. Leiomyomata are much less frequent in the intestine than in the stomach of man and, in the former site, tend to be malignant with about twice the frequency of the gastric tumours (Golden and Stout, 1941; Evans, 1956).

Probably, the absence of numerous mitotic figures in a leiomyoma is not a very reliable guide as to its non-malignancy (McFarland, 1935; Smith, 1937; Evans, 1956), though the relative infrequency of cells with tapered nuclei and scanty cytoplasm provides a further criterion. Thus, although perhaps it is still too early to dogmatize with regard to the malignancy of the canine tumour recorded here, its well circumscribed nature, lack of infiltrative properties, and histological appearance, together with

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PLATE 1

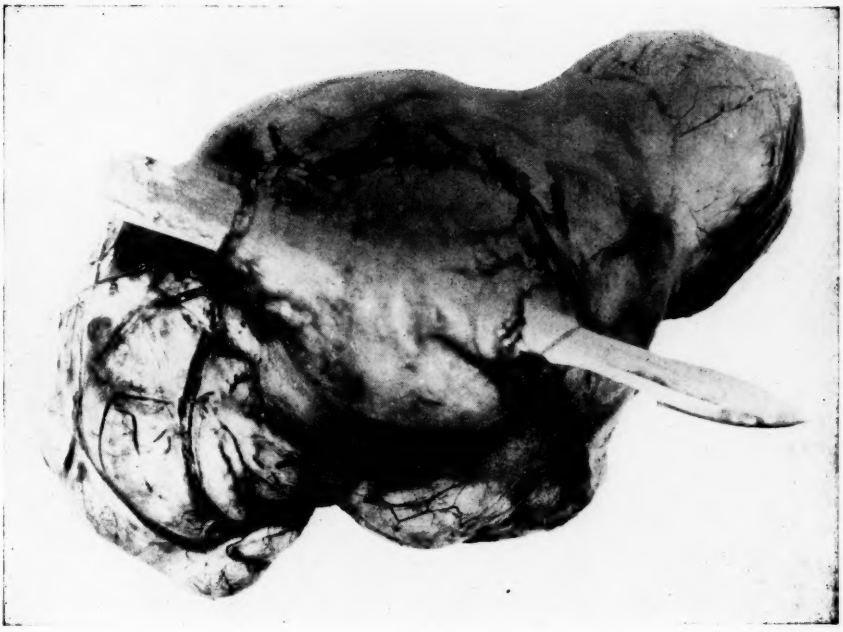

FIG. 1.
Leiomyoma arising from the intestinal wall, showing lobulated appearance and peritoneal covering.

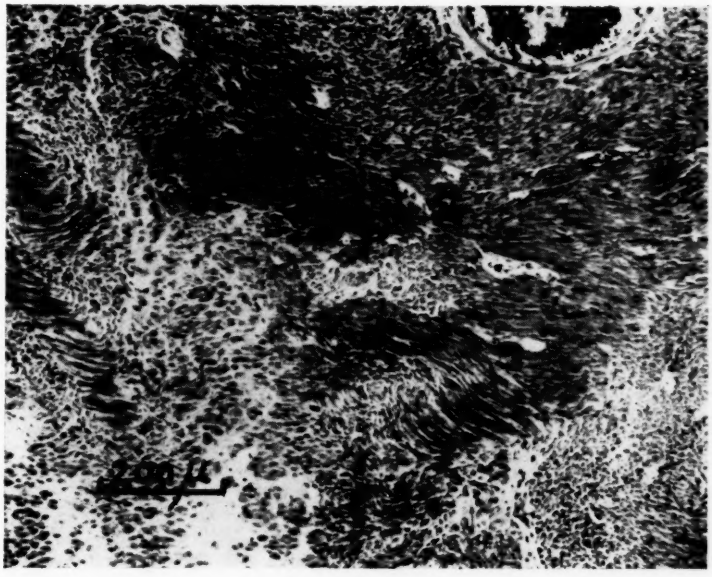

FIG. 2.
Leiomyoma showing muscular and collagenous zones.
Masson's trichrome stain x 90 (approx.)

(Article by N. St. G. Hyslop on pages 156-159)

PLATE 2

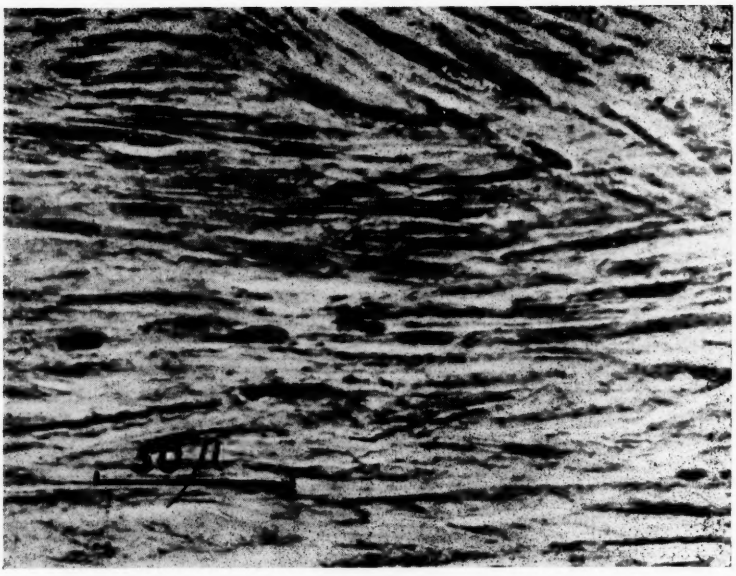

FIG. 3.
Leiomyoma, showing rod-shaped smooth muscle nuclei and fibrils.
Masson's trichrome stain x 600 (approx.)

FIG. 4.
The Bitch, 258 days after extirpation of the tumour.

(Article by N. St. G. Hyslop on pages 156-159)

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the absence of visible metastases at the time of operation all tend to indicate that this particular tumour was benign. This view is supported by the subsequent history of recovery of the dog.

Consideration of the somewhat meagre case histories available suggests that, although similar tumours may often become malignant in human beings, leiomyomata in dogs are almost always benign. The only known exception to this generalization is the leiomyosarcoma recorded by Cotchin (1954).

With reference to the present case, having regard to the relatively short life-span of the dog, the non-recurrence of the tumour during the eight months since its surgical removal probably is equivalent to some five years survival in man.

Summary

A subserous leiomyoma was extirpated from the small intestine of an Alsatian bitch. The tumour did not recur and the bitch remained healthy for eight months.

The histopathological structure of the leiomyoma was described.

Such tumours occur occasionally in human beings but are rare in the dog.

Acknowledgment

We wish to thank Dr. P. M. Biggs for his assistance with the microphotographs, and Mr. P. R. Chivers, who referred the animal to us. We also wish to acknowledge the invaluable technical assistance of Mr. D. Hodgson.

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CONGENITAL PORPHYRIA IN PIGS

By

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Congenital porphyria is well-known in man. It has been described in cattle (Fourie, 1936, 1939) and swine (Clare & Stephens, 1944), but the condition is very rare. In cattle is has been studied in living animals by Fourie and the only investigation of the disease in living pigs is reported in a brief preliminary communication by Clare & Stephens.

Towards the close of 1951 and during most of 1952 congenital porphyria was found to occur rather frequently among the pigs at Thisted slaughterhouse.

The condition is also known as osteo-haemochromatosis, a misleading name, but nevertheless widely employed in the literature. Because of the want of knowledge of this condition in pigs it was decided to make an investigation. The results of this study are reported together with a brief review of the chemistry and biology of porphyrins and a few observations on porphyria in living cattle.

Brief Survey of Porphyrin Chemistry

Our knowledge of the biochemistry of porphyrins has expanded rapidly during the last decade, and several reviews have been published in recent years, the most important being those of Rimington (1936, 1952, 1955), With (1949, 1954), Watson (1951), Vanotti (1954) and the Biochemical Society Symposium (1954). As veterinary surgeons cannot be expected to have an adequate knowledge of porphyrin chemistry, a brief review is included.

Porphyrins are cyclic tetrapyrrolic substances derived from the mother substance porphin (Figure 1) by substitution of various radicals in the positions 1-8. Porphin is not found in nature but it has been synthesized. It consists of 4 pyrrol rings connected by means of 4 methyne bridges ($-CH=$). As seen from Figure 1, two formulae exist, but these formulae do not correspond to isomer forms because the compound—and the same is the case with the porphyrins—shows so-called resonance or mesomerism. This may be explained in the following way: The porphyrin ring constitutes a highly resonating system and the two formulae in Figure 1 are the two limiting configurations of this system. The structure of the actual compound is somewhere between these configurations with the resonating electrons in positions corresponding to the highest degree of stability and the lowest degree of energy. In the literature both the formulae given in Figure 1 are used, but actually neither of them are correct, since porphin and the porphyrins behave as highly resonating systems.

The porphyrins are derived from porphin by substitution of various radicals, most frequently methyl ($-CH_3$), ethyl ($-C_2H_5$), vinyl ($-C_2H_3$), acetic acid ($-CH_2COOH$) and propionic acid ($-CH_2CH_2COOH$). In aetioporphyrin, which does not occur in nature, but has been synthesized, the substituents are 4 methyl groups (in fig. 2 abbreviated M) and 4 ethyl groups (in fig. 2 abbreviated E). As seen from fig. 2 there are four isomeric forms known as aetioporphyrins I-IV. Aetioporphyrin is tetra-methyl-tetra-ethyl-porphin.

The formulae of the porphyrins are most conveniently deduced from the four aetioporphyrins, but only compounds derived from I and III have hitherto been found in nature, and as both are found in animal products Fischer spoke of a " dualism of porphyrins ". This is important because porphyrins of type I and type III cannot be transformed into each other without an opening of the ring, re-arrangement of its pyrrole nuclei, and formation of a new porphyrin ring, and consequently the I and III isomers must be synthesized independently.

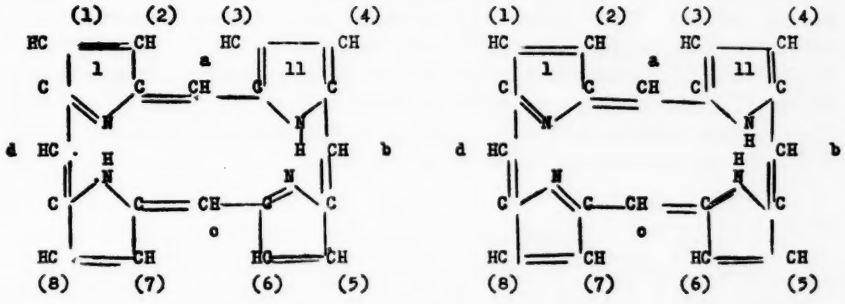


FIG. 1.

The two isomer forms of porphin. The pyrrol rings are designated I-IV, the methyne bridges a-d, the H-atoms 1-8.

By replacing the four ethyl groups in aetioporphyrin by propionic acid groups one obtains tetra-methyl-porphin-tetra-propionic acid called coproporphyrin (fig. 5). Four isomers are known conforming to the same skeleton as aetioporphyrin (fig. 2). It was originally found in faeces, giving rise to the name, but it does also occur in urine. If also the methyl groups of aetioporphyrin are carboxylated (i.e. transformed to acetic acid) one obtains porphin tetra-acetic acid tetra-propionic acid or uroporphyrin (fig. 6), here also the isomers I-IV are known. Uroporphyrin occurs normally in certain species of animals; in man and the domestic animals it is only found as traces under normal conditions, but it is excreted in quantity in certain congenital errors of metabolism known as porphyrias.

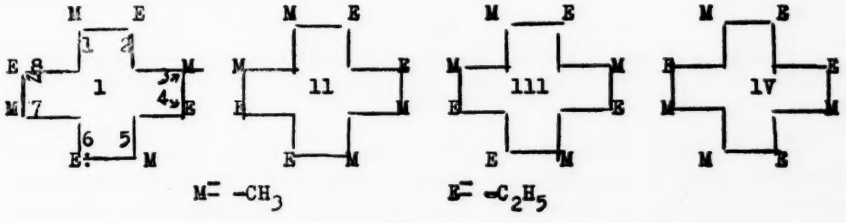


FIG. 2.

The four aetioporphyrins.
Only porphyrins derived from I and III occur in nature. The only difference between I and III is that M. and E, positions 7 and 8, are interchanged.

If all the four methyl groups in aetioporphyrin and two of its ethyl groups are unchanged and only two ethyl groups are carboxylated (i.e. transformed to propionic

acid) the result is tetra-methyl-di-ethyl porphin di-propionic acid, known as mesoporphyrin, and of this compound 15 isomers: I-XV are possible, but only the type IX derived from aetioporphyrin III occurs in nature. If the two ethyl groups in mesoporphyrin are changed to vinyl, one obtains the protoporphyrins: tetra-methyl, di-vinyl porphin, di-propionic acid (fig. 3). Here also XV isomers are known, but only protoporphyrin IX is found in nature, where it occurs in the heme of hemoglobin. Haematoporphyrin is derived from protoporphyrin by substitution of oxyethyl ($-CHOHCH_3$) for vinyl ($-CH=CH_2$). By breakdown of heme with strong acids haematoporphyrin is formed, while protoporphyrin is formed when weak acids or bacteria are used. Other dicarboxylic porphyrins are the deuteroporphyrins (tetra-methyl, di-hydro porphin, di-propionic acid) in which two of the H-atoms of the porphin remain. Deutero- and mesoporphyrins IX may be formed from protoporphyrin IX by bacterial hydration in the intestinal canal and it is found in the faeces. The porphyrin molecule has a discoid form and it shows amphoteric behaviour because of the N in the rings and the COOH in the side chains.

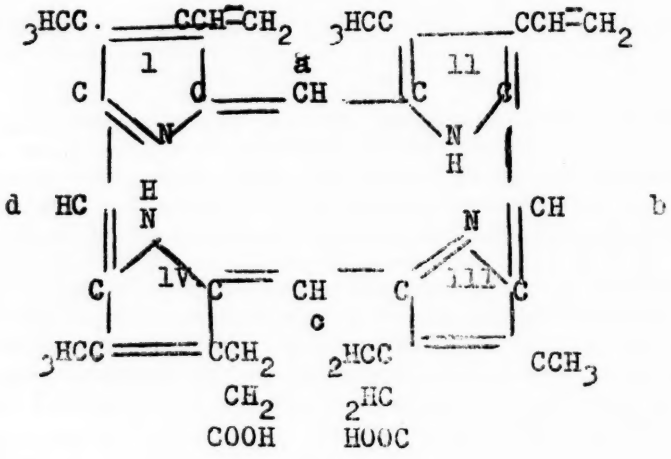


FIG. 3.

Protoporphyrin 1X or 1, 3, 5, 8-tetra-methyl, 2, 4-di-vinyl porphin, 6, 7-di-propionic acid. Protoporphyrin exists in 15 isomer types; only type 1X occurs in nature and is derived from aetioporphyrin 111.

The porphyrins are widely distributed in nature, where they form the active part (prosthetic group) of the respiratory enzymes together with a metal—iron in haemoglobin, magnesium in chlorophyll. Thus porphyrins are fundamental substances necessary for all higher forms of life. They are missing only in the most primitive unicellular forms (the strictly anaerobic organisms). In accordance with this they are formed from the metabolism of the most simple constituents—as has been shown in experiments with " tracer atoms ", *i.e.* the amino acid glycine and simple radicals from the intermediary carbohydrate metabolism such as acetate and succinic acid.

Of the porphyrins found in the respiratory catalysts and oxygen transporters of higher animals mainly one, protoporphyrin, has been studied closely enough to elucidate its structure, but as far as our knowledge go they are all derived from aetioporphyrins I or III.

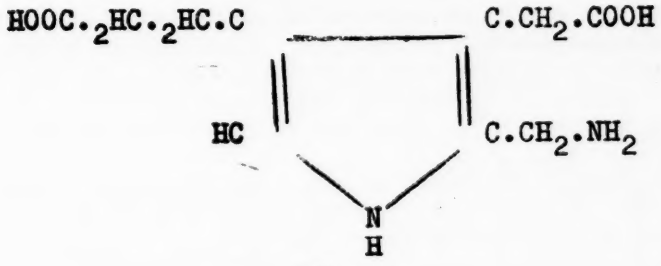


FIG. 4.
Porphobilinogen, the porphyrin precursor

According to the most recent studies all porphyrins are formed from the precusor porphobilinogen, a monopyrrole of the constitution shown in fig. 4. These important studies are summarized in the Biochemical Society Symposia No. 12, 1954 to which the reader is referred for details. Porphobilinogen has been prepared in pure crystalline condition and seems to be the first step in the biological porphyrin synthesis.

The Porphyrias

By *porphyrias* or *porphyrin diseases* we understand inborn errors of metabolism giving rise to pathological porphyrin excretion in urine or faeces or both. Normally only very small quantities of porphyrins are excreted with the urine (in man up to 200 μg. per 24 hours) and faeces (in man 400-600 μg. coproporphyrin per 24 hours). In the urine the main part of the porphyrin is coproporphyrin and only traces of uroporphyrin are present (cf. Comfort *et al.*, 1954, With, 1954, 2). In faeces also, porphyrins from the food and intestinal bacteria (exogenic porphyrin) may occur.

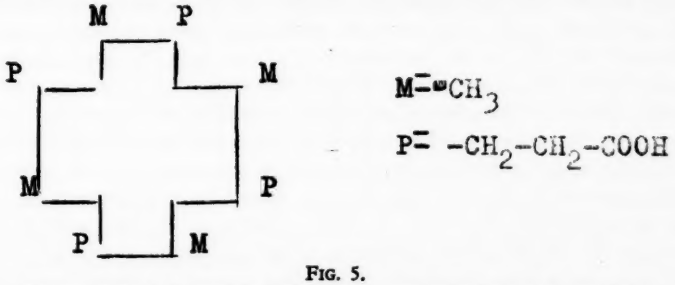
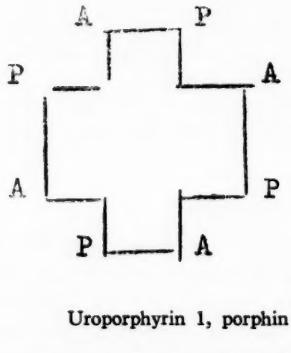


FIG. 5.
Coproporphyrin 1. tetra-methyl porphin, tetra-propionic acid, derived from aetioporphyrin 1.

By *porphyrinuria* we understand an increase of the normal porphyrin excretion in the urine caused by disease, and this is quite different from porphyria which is an inborn error of metabolism (i.e. of the biological porphyrin synthesis).

Porphyrinurias are seen in many pathological conditions: infections, liver injury, intoxications, blood diseases etc., and it is predominantly coproporhyrin which is increased. Until recently it was believed that an increase of the uroporphyrin excretion was only seen in porphyrias—i.e., that uroporphyrinuria was pathognomonic of porphyria—but With (1954, 2) found a small but definite, increase of uroporphyrin and other pathological porphyrins in certain diseases, so this hypothesis cannot be maintained. But excretion of uroporphyrin in quantity—several milligrammes per 24 hours—is pathognomonic of porphyria.



$P^{=}$ $-CH_2-CH_2-COOH$

$A^{=}$ $-CH_2-COOH$

FIG. 6.

Uroporphyrin 1, porphin tetra-acetic acid, tetra-propionic acid.

The porphyrias are subdivided into: (1) *acute porphyria,* (2) *congenital porphyria,* and (3) *porphyria cutanea tarda,* cutaneous porphyria starting later in life.

Acute porphyria is known only in man. It can be produced experimentally in small laboratory animals (rabbits, rats, chickens, Schmid & Schwartz, 1952; Goldberg, 1954; Rimington, personal communication 1955) by certain intoxications. It has not been described in domestic animals. It is not an acute disease in the ordinary sense, but a permanent metabolic error with intermittent manifestation and quiescent periods—so-called latent periods or latent porphyria. The manifest attacks are located in 1. the *gastro-intestinal system* (abdominal cramps simulating " acute abdomen "). Often the patients are operated upon under the erroneous diagnosis of acute appendicitis or intestinal obstruction; 2. *the nervous system,* widespread motor and sensory pareses are exhibited which may involve the respiration and thus be fatal and finally, 3. *psychoses.* The disease is often lethal, especially if the nervous system is involved, and post-mortem examination shows only insignificant anatomical alterations of the organs. The most important metabolic error in this condition is the excretion of *porphobilinogen* in quantity in the urine. The porphyrins found in the urine are all formed from porphobilinogen—either after the urine has been voided, or in the urinary passages. No porphyrin is deposited in the bones or other organs. In typical cases the patients never develop skin affections due to light as in the case with the chronic porphyrias, but rare cases have been described of so-called mixed porphyria combining the cutaneous symptoms of chronic porphyria with the symptoms of acute porphyria.

Chronic porphyria is a condition of quite another nature. It is—in human pathology—most often subdivided into a congenital form and porphyria cutanea tarda. In congenital porphyria no porphobilinogen excretion is found, but the porphyrins them-

selves are excreted in large amounts in the urine and faeces (bile), and deposited in the bones and teeth. In severe cases porphyrins can be demonstrated in the blood serum. In the urine both uro- and coproporphyrins are excreted, in the faeces copro- and protoporphyrins and only rarely uroporphyrin. The porphyrin deposited in the bony structures is uroporphyrin.

One of the most characteristic symptoms of chronic porphyria in man and cattle is *increased light sensitivity of the skin* giving rise to light-dermatoses on the most exposed parts of the skin. In pigs these dermatoses have not been observed. In cattle generally they are not very pronounced, and according to our observations in Denmark they occur only in places where the skin is free from pigment. In man, however, these light dermatoses may be very violent and cause mutilation of the face and hands.

In severe cases, especially with manifestation early in life, a chronic anemia of the haemolytic type accompanied by plenomegaly is manifested. As a rule the bone marrow of cattle with porphyria is found to be hyperplastic at autopsy.

The symptoms of congenital porphyria vary very much in intensity from case to case both in man and the domestic animals, the milder cases are practically free from clinical signs and symptoms, except for the pigmentation of the teeth and bones.

Porphyria has never been described in horses, or in wild animals.

The porphyrins excreted in the porphyrias are not only uroporphyrins (8 carboxyl groups) in urine, very rarely in faeces, and coproporphyrins (4 carboxyl groups) in urine and faeces, but any porphyrin with between 1 and 8 carboxyl groups may occur. These porphyrins are found also in extremely small amounts in normal urines (Comfort & Wetherall, 1954) and in somewhat greater amounts in the symptomatic porphyrinuria accompanying certain disease (With, 1954).

Theories of the Porphyrin Metabolism in the Porphyrias

Several theories of porphyrin metabolism have been suggested, and since the introduction of labelling with isotopes in biochemical research a considerable amount of information has been assembled. The studies of Shemin and Rittenberg and their associates in U.S.A. and Neuberger, Gray and their associates in England have contributed in some measure to the elucidation of these questions. Only a brief outline is given in this paper, more information may be found in the surveys quoted above and in the recent brief review of Muir (1954).

In the older literature *two rival theories* are discussed: the theory of *synthesis* and the theory of *disintegration*. According to the former the pathological porphyrins in porphyria are products of an abnormal porphyrin synthesis, and according to the latter they are formed as by-products of an abnormal disintegration of the blood-pigment hemoglobin. Thanks to the above mentioned experiments and studies with labelled atoms (especially feeding experiments with N^{15}-labelled glycine and C^{14}-labelled acetate) it is known that the *synthesis theory is right* and the *disintegration theory is wrong*. The small amounts of porphyrins excreted under normal conditions are by-products of the hemoglobin (protoporphyrin-) synthesis and not of hemoglobin disintegration. Porphobilinogen is probably the precursor of all porphyrins, both normal and pathological, and the porphyrias are due to anomalies in the enzymatic systems governing the transformation of porphobilinogen into protoporphyrin. In acute

porphyria a considerable part of the porphobilinogen escapes conversion and is excreted, and in the chronic porphyrias it gives rise to large amounts of other porphyrins—especially of the uro- (8 carboxyl groups) and the copro- (4 carboxyl groups) types—as well as the protoporphyrin (2 carboxyl groups). These other porphyrins are also formed under normal conditions, but only in minute amounts. The pathological porphyrins in the porphyrias are mostly type I isomers (cf. above), but also substantial amounts of type III are formed (e.g., 85% type I and 15% type III). Contrary to this *the protoporphyrin of haemoglobin* (protoporphyrin IX) is a type III derivative.

In spite of the important progress of the last decade our knowledge of the biochemical lesion in the porphyrias is still very limited. Watson and his School are of the opinion that the lesion of acute porphyria as well as that of the cutanea tarda type is located in the liver (hepatic porphyria) while the cause of congenital porphyria is to be sought in the haemoglobin synthesis in the bone marrow.

Congenital Porphyria in Pigs

In animals congenital porphyria has been described only in cattle where it was found to be hereditary, apparently depending on a recessive gene (Fourie 1936, 1939; Rimington, Roest & Fourie, 1938; Fourie & Rimington, 1938) and in swine in a very brief preliminary notice (Clare & Stephens, 1944). Clare & Stephens described—from New Zealand—a sow giving birth to three litters of pigs with discoloured bones and by two different boars. The boars were closely related (half-brothers with the same father). A whole-sister of the sow was mated with one of the boars and gave birth to a litter of pigs all of which showed normal colour of the bones. The sow excreted porphyrins in both urine and faeces. On clinical examination both the boars appeared to be normal. The pigs of Clare & Stephens did not show photosensitivity.

When the disease was discovered *among the swine at Thisted* the first object was to study factors appertaining to the *heredity nature of the condition.*

The Material

It was soon found that *all cases* among pigs at Thisted could be *traced to a certain boar.* Either this boar was the father of the affected pigs—with discoloured teeth and bones—or the mother was a daughter of the boar showing discoloured teeth and bones. Before this particular boar was introduced into the pig-breeding in Thisted county discolouration of teeth and bones in pigs had not been observed at the slaughterhouse.

In the beginning the boar was used together with a second older boar in a total of 56 matings giving rise to eight litters with congenital porphyria. Later the boar was used alone in 171 matings of which at least 49—and possibly 53, as in four cases there were mixed litters—gave litters with congenital porphyria. In no case were the sows of the parental generation found to suffer from porphyria although several of them were subjected to special examination for porphyria. The teeth of all the sows employed for breeding had been inspected a few years before in order to determine

their age. It is reasonable to suppose that if some of them had suffered from congenital porphyria the discolouration of their teeth would not have been overlooked.

In the first and second filial generation porphyria was demonstrated in 141 cases, one of them a pig dying one hour after birth. Of the 141 cases 65 were females, 66 males and 10 of unknown sex. As soon as the consequences of employing the boar in the breeding became clear the farmers were warned against its use, and the material is therefore limited to the first and the second filial generation and some sows of the first filial generation.

In three litters of sows from the first filial generation pigs with porphyria have been found, and two of the sows have been identified and showed to suffer from porphyria. The third sow could not be identified. The mating of these three sows was performed by the porphyria-boar only in one case. In the two other cases the mating was by normal boars. The offspring of these two normal boars with normal sows, including sows from the parental generation who earlier gave birth to pigs with congenital porphyria together with the porphyria-boar, did not exhibit discoloured teeth and bones. In one case one apparently normal sow—a daughter of the porphyria-boar—was mated with a normal boar, and all the offspring were normal. Two sows from the first filial generation — whole-sisters and named Thisted I and II — were transferred to the State Agricultural Experiment Station of Faverholm for use in systematic breeding experiments.

On nine occasions it was possible to study all the pigs in the litters whilst they were alive and after slaughter.

Number of litter	Size of litter	Number of pigs in the litter with porphyria
1	7	5
2	7	3
3	3	3
4	9	5
5	11	2
6	7	4
7	9	7
8	4	2
9	8	2

Litters No. 3 and 5 were from the second filial generation, the others from the first.

It is seen from the material presented that the condition is inherited both through boars and through sows, since sows with the condition from the first filial generation were able to transmit the disease with normal boars.

XUM

Chemical Investigations

The chemical studies were carried out on bones, teeth, urine and blood from the animals by E. Nielsen, M. D. and E. Bierring, M. D. of Medicinsk Laboratorium, Vognmagergade, Copenhagen. The technique consisted of inspection in Wood's light to discover the characteristic red fluorescence of porphyrins. The blood and urine were subjected to extraction with ether-acetic acid—which extracts the coproporphyrins—and subsequently to extraction with ethyl acetate at pH about 3—to extract the uroporphyrins. If the extracts showed red fluorescence they were treated with 25% hydrochloric acid and the hydrochloric acid extract was subjected to spectrophotometric study in a Bechmann spectrophotometer. After extraction with acetic acid ether and ethyl acetate no fluorescence remained in the blood or urine in any of the cases studied.

Chemical Studies of Teeth and Bones

Qualitative analysis only was carried out by inspection under Wood's light. Later With (1955) elaborated a technique for quantitative analysis and studied the offspring of the two sows Thisted I and II.

Bones and teeth from normal pigs—adult as well as new-born—never showed a red fluorescence in Wood's light. Discoloured teeth—both from newborn and older pigs—always showed the characteristic red fluorescence.

Later With (1955) found that even very dark teeth from newborn pigs may fail to show fluorescence, but in these cases extracts of such teeth with dilute hydrochloric acid always showed a beautiful red fluorescence. In some cases teeth and bones from normal newborn pigs may exhibit a very weak, but quite definite, red fluorescence of their extracts with dilute HCl. (Unpublished findings of T. K. With). This quite minute deposition of porphyrins in the teeth and bones of some normal newborn pigs is without connection with congenital porphyria and may be due to coproporphyrin.

The skeleton and a piece of skin of a suckling pig suffering from congenital porphyria was studied after being stored for 9 months and loosing much of its original red colour. The crowns and columns of all the incisor teeth fluoresced strongly red. The molar teeth did not show any fluorescence. The lower part of the mandible, many parts of the cranium as well as parts of the long bones adjacent to the epiphyseal lines and some of the vertebrae showed distinct red fluorescence. The remainder of the skeleton and the piece of the skin did not show any fluorescence. The femurs and lower jaws with teeth of two pigs with slight discolouration of the bones (numbers 7173/1 and 7173/2) were subjected to fluorescence analysis when in a fresh condition. Their blood was studied also but did not show any fluorescence. All the discoloured teeth showed pronounced red fluorescence and the jaw and thigh bones exhibited patches of red fluorescence. The fluorescence was intensified after removal of the periosteum. The cartilage showed no red, but a clear blue fluorescence.

With (1955) performed quantitative analysis of bones and teeth of the same line of pigs. He found up to 200 microgrammes per g. in the teeth of newborn pigs with porphyria and by means of paper chromatography he showed that the main part

of the porphyrin of the bones and teeth of the pigs was uroporphyrin although some coproporphyrin was present also. The main part of the bone-porphyrin was found to be of type 1, but some type III porphyrin (uroporphyrin III) was present also (cf. Jorgensen & With, 1955).

Chemical Studies of Urine

The content of the urinary bladder was collected after the slaughter of the animals. The 24-hour urine was not studied, but later With analysed 24-hour urine samples of other pigs from the same line (cf. Jorgensen & With, 1955).

As a control experiment the urine of 5 normal pigs as well as from 3 apparently normal pigs from litters in which some of the pigs exhibited porphyria were subjected to analysis. No porphyrin could be demonstrated in any of these urines.

The urine of eleven pigs with porphyria but without disturbance of their general condition was studied. Porphyrin was demonstrated in only four of these urines. The findings in these four cases were as follows:

Pig No. 1324A, exhibited strong discolouration of the bones. The urine was definitely reddish and both coproporphyrin and uroporphyrin could be extracted. The HCl extract of the ether phase showed maxima 4050, 5500 and 5940A, that of the ethyl acetate phase showed maxima 4110-4120, 5540 and 5940A.

Pig No. 1324C, exhibiting medium discolouration of the bones and a reddish tint of the urine. The findings were as in pig No. 1324A. In both these cases large amounts of porphyrins were present.

Pig No. 1548/11, with below medium discolouration of the bones. No uroporphyrin, but a little coproporphyrin was demonstrated (6,5 microgrammes coproporphyrin per 100 ml. urine).

Pig No. 1324/15. Only slight discolouration of the bones. The urine was yellow without reddish tint. Small amounts of both coproporphyrin and uroporphyrin were demonstrated.

The seven pigs with porphyria where no porphyrin could be demonstrated in the urine exhibited either slight or medium discolouration of the bones, and the urine was in all cases light yellow.

These observations show that there is no close correlation between bone discolouration and urinary porphyrin excretion. Porphyrins may occur in the urine even in cases with slight bone discolouration.

In studies of the descendants of the Thisted pigs of Faverholm With found a 24-hour excretion of 1,000-10,000 microgrammes uroporphyrin in the severely affected pigs and 100-750 microgrammes in the slightly affected; the coproporphyrin excretion was considerably lower, amounting only to about 50 microgrammes per 24-hours in the severely affected. Paper chromatography showed that in addition minute amounts of porphyrins with 3, 5, 6 and 7 carboxyl groups were present and that the main portion of the uroporphyrin and coproporphyrin was of type 1 (cf. also Jorgensen & With, 1955). No porphobilinogen could be demonstrated. The urinary excretion of porphyrins was found to be intermittent as some pigs showing high excretion of both uro- and coproporphyrins at the age of a few months showed only coproporphyrin about one year later and that in a rather low concentration in their urine.

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Chemical Studies on Blood

Eight pigs with porphyria were studied and porphyrin was found in the blood of two of them.

Pig. No. 13356 (1. filial generation) with strong bone discolouration and designated as sick on inspection before it was slaughtered. Both the untreated serum and the de-proteinized filtrate exhibited strong fluorescence. The HCl extract of the ether phase showed maxima 4.000-4050A and that of the ethyl acetate phase 4050A.

Pig No. 21844 (second filial generation) with strong bone discolouration and disturbed general condition showed the characteristic porphyrin fluorescence in both plasma and erythrocytes. Owing to the limited amounts of material further analyses could not be carried out.

Symptoms and Diagnosis of Congenital Porphyria in Pigs

The symptoms of congenital porphyria in pigs are surprisingly few. Apart from the two pigs with porphyrin in the blood which exhibited disturbed general condition no symptoms were observed except the discolouration of teeth and bones and reddish urine in some cases. Photosensitivity and dermatoses were not reported. Similar observations were made when the offspring of the Thisted pigs at the State Experimental Farm of Faverholm were studied. In these instances only one pig exhibited a disturbed general condition and no case of photosensitivity was observed. This is in good agreement with the findings of Clare & Stephens whose pigs were of a black race. Our pigs were of the white Danish race but nevertheless did not show any photosensitivity.

The discolouration of the teeth is definitely progressive, a fact readily demonstrated by study at intervals of the teeth of a growing porphyria pig. The discolouration is plainly visible on the canine teeth of the newborn pigs in most cases, and as a rule it is most pronounced on the posterior surface of the incisor teeth. All black teeth do not exhibit the characteristic fluorescence, but extraction of the teeth with 0.5 N hydrochloric acid and subjecting the extract to inspection in Wood's light will indicate rapidly whether the pig suffers from porphyria (With, 1955). The porphyrins are located in the dentine, and the enamel is free from fluorescence.

The discolouration of the teeth is very characteristic, and even without the aid of fluorescence analyses the diagnosis can be made with a high degree of accuracy. In order to study the diagnostic value of discolouration of the teeth 10,000 normal pig skulls with teeth were inspected. The result of this study may be summarized as follows.

I. In no case did the discolouration like that found in congenital porphyria occur in normal adult pigs.

II. A diagnosis based on inspection of the teeth in vivo was never found to be wrong when the pig was slaughtered although 2-2½ months elapsed between the diagnosis and the slaughter.*

III. In the period when the colour of the teeth was subject to systematic study discolouration of the bones was never found unless discolouration of the teeth had been observed in the same animal.

* Later With *et al.* (1959) found in some cases that pigs with definitely discoloured teeth at birth were normal when slaughtered 6 months later.

IV. There seems to be a certain degree of proportion between the discolouration of the bones and that of teeth, but, as a rule, in mild cases the discolouration of the teeth is more pronounced than that of the bones.

V. The colour of the teeth is not quite identical in the living and the dead pig. In the former the colour is warm and red, while in the latter it is more brown. If the bones are stored the colour fades gradually.

VI. In the great majority of cases discoloured teeth and bones from pigs with porphyria will give beautiful fluorescence in Wood's light. The diagnosis of porphyria, however, cannot be entirely excluded if a discoloured tooth or bone fails to fluoresce with a red colour in Wood's light. According to With (1955) porphyria can only be excluded in such cases by grinding tooth or bone to a fine powder and extracting the latter with 0.5 N HCl. If this extract is free from red fluorescence porphyria can be excluded.

VII. The discolouration of the teeth of cattle affected with porphyria is very characteristic and is distinguished easily from the discolouration seen in normal cattle. The presence or absence of fluorescence in Wood's light is a valuable aid in cases of doubt. While the discolouration was always present in the teeth of the newborn pigs this is not the case in the newborn calf, Fourie (1936) says that the teeth may be normal at birth and become discoloured later. One of the calves in our breeding experiment showed for the first time a quite evident discolouration of the teeth on the third day after birth.

In the diagnosis of porphyria—both in pigs and cattle—the study of urine, faeces and blood is also of value. Demonstrable amounts of porphyrin in the blood have been found only in the most pronounced cases. In swine the excretion in the urine seems to be intermittent, and the faecal excretion of porphyrins may be small even in cases of well pronounced porphyria.

Hereditary Transmission

We found that a boar, with discolouration of the teeth and bones, transmitted the condition to his offspring when mated to normal sows. Further his female offspring transmitted the condition to their offspring when mated to normal boars, provided that they themselves showed discolouration of teeth and bones. In no case did the offspring of porphyria pigs which were free from discolouration transmit the condition to their offspring when mated with normal boars.

Discolouration of the teeth in congenital porphyria is found in cattle as well as in pigs. I have had the opportunity to study about 40 cows, bulls and calves with congenital porphyria (cf. Jorgensen & With, 1955), and also Fourie and collaborators noted the colour of the teeth in cattle with congenital porphyria. The diagnostic value of the colour of the teeth in cattle is not equal to that in swine because cows and bulls with normal teeth can transfer the disease to their offspring while, according to our observations, pigs with normal teeth never do so. There seems to be an important difference between the heriditary transmission of porphyria in pigs which is apparently dependent upon one or more dominant genes and that in cattle which seems to depend on a recessive factor, according to the studies of Fourie and associates in South Africa which are supported by our observations in Denmark (cf. Jorgensen & With, 1955).

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As porphyria in pigs—and in cattle too—may be transmitted both through males and females, sex-linkage is out of the question.

The disease may depend on a single pair of genes in pigs, but it seems more probable that more than one pair of genes are involved, and presumably genes linked to each other, because not only porphyrins, but also at least one other dark colouring matter, is deposited in the teeth and bones and the amount of this matter shows a certain, but far from absolute, parallelism with the porphyrin with regard to its deposition in bones and teeth. The histological findings also support the view that at least two pigment systems are involved.

That porphyria in pigs can hardly be transmitted by means of a recessive gene is understood easily. If that hypothesis be correct about 30% of the apparently normal sows of the parental generation must have possessed this gene to give the frequency of the condition found in the first filial generation. The fact that the two normal boars produced offspring with porphyria with porphyria-sows from the first filial generation, and only gave normal offspring with sows from the parental generation which had produced porphyria pigs when mated with the porphyria boar speaks strongly against the possibility of transmittance with a recessive gene.

The assumption that the disease is inherited with one or more dominant genes is not contradictory to the few observations of Clare & Stephens, and the continued observations at Faverholm with the sows Thisted I and II are also in agreement with this view (With *et al.*, 1959).

Histological Studies

These studies were completed before it became clear that the condition was congenital porphyria, consequently the studies did not comprise fluorescence microscopy. The histological examination was carried out by Prosector W. Kiær, M.D., of the Central County Hospital of Svendborg, Denmark. In the beginning of our investigations we had diagnosed the condition as "osteohæmochromatosis" without knowing that this disease in swine is identical with congenital porphyria, and consequently the histological studies were principally directed towards the finding of similarities with or differences from haemochromatosis in man.

All the specimens were fixed with ten per cent. formalin and the bones and teeth were decalcified with formic acid-sodium formate. Later studies by With (personal communication) have shown that the porphyrins are extracted from the bony tissues during this decalcification procedure. Paraffin sections were prepared in the usual way with all the tissue samples. The stainings used as standard methods were haematoxylin-eosin and staining according to van Gieson-Hansen. As supplements a modified Berlin-blue reaction—with heated HCl—performed in order to demonstrate iron-containing pigment, a reaction for melanin (Fontana's silver solution), and fat staining with Sudan III. The reliability of the staining methods was controlled with human tissue samples.

The material studied consisted of the following organs from a pig with a slight degree of congenital porphyria (No. A 1287): skin, bones, teeth, ear cartilage, lung, liver, spleen, heart, pancreas, lymph nodes and muscles. The same organs from two

other pigs with porphyria (No. B 7173 and 1278, the latter with severe porphyria) were also subjected to histological study, and in these instances the kidneys were included also. The same organs from a normal pig were included as control.

In the pig with slight porphyria (A 1287) the skin, ear-cartilage, lung, liver, spleen, teeth, heart, lymph nodes, voluntary muscle and the pancreas showed no deviations from the normal control preparations. There was no abnormal pigment depositions, no cirrhosis of the liver or pancreas, no fibrosis, and no extra-medullary haematopoiesis. The bone-marrow was normal but the bone itself showed a picture resembling early osteo-myelosclerosis. This picture was not found in the other pigs with porphyria, and as similar pictures are found occasionally in man as an accidental finding, this myelosclerosis-like picture is most probably an accidental finding. There were also some rather big cells, containing pigment granules which were coloured brownish with haematoxylin-eosin.

The porphyria pig B 7173 showed bone tissue without histological abnormalities and negative pigment reactions. Only the bone was studied.

The pig with severe porphyria (No. 1278) showed normal cartilage, skin, musculature (voluntary and heart) and lymph-nodes. The pancreas sample was missing. The *liver* exhibited a well-marked infiltration with small dirty-yellowish pigment granules in the great majority of the Kupffer cells, but these cells were not deformed or enlarged. The parenchymal cells showed only minimal pigmentation in a few places, and the interstitial tissue was normal. The iron reaction was positive, and in most places strong, in some places this reaction, however, was weak or even negative. The iron reaction showed variation, even within the same cell. The silver reaction (melanin) was in some places positive, which must be looked upon as normal. Apart from the pigment and the iron reaction no difference from the normal control was demonstrated, especially, there was no fibrosis or other signs of cirrhosis. The *spleen* pulp contained numerous star-shaped or angular cells of medium size, resembling reticulum cells. These cells contained the same pigment granules as found in the stellate cells of the liver. These granules have a strongly positive iron reaction. As in the liver the melanin-silver reaction was positive in some places. Some very large cells with a single large, dark and angular nucleus were scattered about, these cells were pigment-free and without relation to the iron-containing cells. Apart from those mentioned no deviations from the normal control were demonstrated in the spleen. The *kidney* showed in a few of the convoluted tubules, a slight pigmentation of the same nature as in the liver. The *lungs* exhibited numerous small to medium pigment-containing cells in the alveolar septa. The iron reaction was strongly positive in all places. The alveolar septa did not show thickening. The silver-melanin reaction was negative. The marrow cells looked normal, but scattered among them numerous large, irregular cells were found. They were rich in protoplasm with ill-defined cell-limits and comparatively small nuclei. Their protoplasm contained numerous relatively fine brownish or dirty-yellowish pigment granules. The iron reaction was completely negative, but the silver reaction was fairly strong as found in the presence of melanotic pigment, yet a number of them did not blacken. No fibrosis was found in the marrow. The spongy bone and the cartilage were normal with the standard staining and the iron reaction was negative in both, whereas the melanin reaction was positive in scattering stained areas in the spongy bone. Compact bony tissue showed essentially

the same picture as the spongy bone, with the exception that the marrow was less cellular. The *teeth* were very difficult to make sections from, the dentine and enamel did not show a significant difference from the normal control, but the adjacent bone tissue showed the same alterations as described under bone.

In *conclusion* a pigment anomaly located in liver, spleen, kidney, lungs and bone tissues was found, but apart from the pigment deposition no anomalies could be demonstrated. According to the behaviour of the pigment granules towards the different histo-chemical reactions at least two different types of pigment are involved. The studies did not lead to any definite conclusion, but there is no similarity to haemochromatosis. It may be added that quite similar findings in porphyria in cattle have been reported by Poulsen (1910) and Moller-Sorensen (1920).

Pathology (Macroscopic)

Skin: the deeper layers were normal. The epidermis was not studied in the dead animals as it was removed during the slaughter, but in the living animals it was normal. No skin biopsies were performed. *Musculature*: normal, occasionally a slight yellow tint. *Cartilage*: normal. *Vertibrae*: increased porosity, *the intervertebral discs* were normal, with the exception that their nuclei were occasionally discoloured (dark). *The long bones*: showed increased porosity both in the compact and spongy parts. *The bone marrow* was often definitely darker than normal (red) and often showed evidence of bleeding between the bony wall and the marrow tissues. *The glands* were often yellowish-brown on the cut-surface. *The lungs* were normal to yellowish-brown in colour. *The mucous membrane* of the trachea was normal to slightly cream coloured. *The tracheal cartilage* was normal. *The heart and the great vessels* were normal in colour to yellowish-brown. *The liver and the kidneys* were normal in colour to dark-brown. The *spleen* was normal. The *intestines* were normal to yellow in colour. The *urinary bladder* was normal. The colour of the bones varied from almost normal to dark brown. The periosteum was normal.

Summary

An account is given of the occurrence of congenital porphyria in pigs in a Danish county. All the cases could be traced to a single boar.

The condition is heriditary and depends on one or more dominant genes, contrary to congenital porphyria in cattle which depends on a recessive gene.

The principal signs of the disease is discolouration of the teeth and bones, and this makes the diagnosis possible in newborn pigs. The general condition of the animals was only affected in a few cases, and light sensitivity of the skin was not observed.

The pathology—macro- and microscopic—as well as the chemical pathology of the condition are described, and a summary of the present knowledge of porphyrins and porphyrin disease is presented.

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Postscript at the proof: The systematic studies on the offspring of the Thisted pigs at the Danish State Experimental Farm at Favrholm have now been published in Danish with English summary as 312 report from the State Agricultural Experiment Laboratory (312 fra Forsogslaboratoriet, Copenhagen, 1959) by Torben K. With, H. Clausen and N. J. Hojgaard-Olsen.

Acknowledgments

The persons and institutions mentioned below have helped me in different ways with support and encouragement during my work for which it is a pleasure to me to give my heartiest thanks: Chief-Pathologist W. Kiær, M.D. and Chief-Physician Torben K. With, M.D., of Svendborg County Hospital, Chief-Physician Holger Nielsen, M.D., of Thisted County Hospital, Richard Christensen, Physician of Svendborg, Professor Hjalmar Clausen, The Royal Veterinary and Agricultural College, Copenhagen, Professor Rasbech, The Royal Veterinary and Agricultural College, Copenhagen, farmer Jens Kirk, Skinnerup, Thisted. I am greatly indebted to Professor Rimington of University College Medical School, London, and to the editor of *Endeavour* for the use of the colour plates. I thank also the executives of the Bacon Factory of Thisted for their continued interest and assistance.

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XUM

INCIDENCE OF GOITRE IN GOATS AND SHEEP IN INDIA

By

BRAHM DUTT and N. D. KEHAR

Animal Nutrition Division, Indian Veterinary Research Institute, Izat-Nagar (U.P.)

Goitre occurs in human beings and animals. Scientific investigations on the aetiology of goitre began when Halsted (1896) extirpated almost all the glandular tissues from dogs early in pregnancy and found thyroid enlargement in their off-spring. Later Marine *et al.* (1909) proved that congenital goitre could be prevented by the administration of potassium iodide to the dam during gestation.

Smith (1917) was successful in preventing the birth of hairless cretin-like off-spring from certain pregnant sows by feeding iodine. These experiments lent support to the growing idea that iodine deficiency and goitre are closely related. While it has been established that goitre is associated with an iodine deficiency, these facts do not explain its whole pathology.

Infections, intoxications, environmental temperature and excesses in food intake have been considered by various workers to influence the thyroid gland. McCarrison (1914) has adduced striking evidence, from observations in India, of variations in the incidence of goitre in human beings in different districts of the same region, and considers that this is due to bacterial pollution of streams.

McCarrison (1919) also reported that dietary deficiencies in vitamin B accessory food factors lead to a reduction in size and weight of thyroid gland. He (1912) reported also that enlargement of the thyroid gland takes place in vitamin A deficiency. Thompson (1932) reported that hyperplasia of the thyroid glands of albino rats took place when the rats were fed rachitogenic diet (low in phosphorus and high in calcium). He reported that by increasing the content of calcium carbonate in the diet deficient in iodine, the degree of hyperplasia was enhanced. There are few reports about the incidence of goitre in animals in India. Sen (1935) mentions only one instance of goitre in the young calves at the Pussa Institute. This was resolved by the administration of potassium iodide.

Nagina Singh (1946) noted cases of goitre in goats in Manumagra and Kalka in Ambala District (Punjab). The water samples in these areas were found to be totally deficient in iodine.

Gopal Krishnan (1946-47) reported that in the foot hill regions in Assam, it is a common sight to see some people with goitrous swellings of variable sizes in the neck. He reported that though such swelling is not usually observed in cattle, slight enlargement of thyroid glands and attendant clinical symptoms indicate iodine deficiency.

Brahm Dutt and Ray (1955-56) reported that though clinical cases of goitre were not seen in hilly regions of Punjab and U.P. (India), yet histopathological examination of thyroid glands of goats from such areas revealed a hyperplastic condition indicating the prevalence of sub-clinical goitre.

Material and Methods

In order to assess the incidence of goitre in goats and sheep in different age and sex groups, about 1000 thyroid glands were collected from the Bareilly slaughter house during the course of a year. They were weighed and examined histopathologically for changes associated with goitre.

Results

Marked variation in weight of the thyroid glands of goats in different age and sex groups was noticed. There was no appreciable difference in the weight of the thyroid glands of the sheep. An estimate of the variation in the weight of the thyroid gland in different age and sex groups, in goats and sheep can be formed from the figures given below:—

1.	Wt. of the thyroid glands of female goats age one year and over	0.8 — 93.0 g.
2.	Wt. of thyroid glands of female goats age under 1 year ...	0.6 — 19.0 g.
3.	Wt. of thyroid glands of male goats age one year and over	0.6 — 9.0 g.
4.	Wt. of thyroid glands of male goats age under one year	0.5 — 6.5 g.
5.	Wt. of thyroid glands of castrated male goats age 1 year and over	1.5 — 17.0 g.
6.	Wt. of thyroid glands of castrated male goats age under 1 year	0.5 — 9.4 g.
7.	Wt. of thyroid glands of female sheep age 1 year and over	0.4 — 1.5 g.
8.	Wt. of thyroid glands of rams age 1 year and over ...	0.4 — 0.6 g.
9.	Wt. of thyroid glands of rams of full age	0.6 — 1.2 g.

Perusal of the above data leads one to assume the following:

1. There is marked variation in the weight of the thyroid glands in goats as compared with sheep.
2. The variation is more marked in females than in male goats.
3. Variation is more marked in the female goats under one year than in the corresponding group of male goats.
4. Castrated male goats, both in the fully grown group and in the 1 year group, show more pronounced variation in weight of their thyroid glands than the corresponding groups of uncastrated male goats.
5. Sheep irrespective of their age and sex do not show appreciable variation in weight of their thyroid glands.
6. The incidence of goitre is more common in goats, particularly in female goats whilst not a single case of goitre was observed in sheep. About 10 per cent. of the goats were found to be goitrous.

While clinical and sub-clinical cases of goitre were seen in goats, other signs such as dwarfism were not seen in any case of goitre.

Russian workers Jovanovic *et al.* (1954) carried out a survey of enzotic goitre in domestic animals in Siberia. They reported that the incidence of enlarged thyroid glands and the degree of enlargement were greater among goats and that other signs such as dwarfism, sterility and low vitality were not seen, though poor lactation was

reported, in those animals in which the thyroid gland was found to be enlarged. They also reported that enlargement was not so pronounced in sheep but poor lactation and wool production and high mortality rates in lambs were noted.

Histopathological changes

Thyroid glands were fixed in Formol Saline and embedded in paraffin. Sections were cut 5-6 μ thick and stained by Haematoxylin and Eosin.

Goitre met with in sporadic cases in goats was of the parenchymatous type. In thyroid glands, which were moderately enlarged, the normal cells were changed from their flat or cubical form to columnar shape. Thyroid glands which were greatly hyperplastic, exhibited acini lined by tall columnar cells projecting into the lumen of the follicles and thereby the colloid content was diminished. The colloid seen in the acini of hyperplastic thyroid glands was of a nature different to normal. It was watery and stained very lightly as compared with colloid seen in normal glands. Blood vessels were very congested. In some of the acini no colloid was seen (fig. 1), whereas a normal thyroid acinus is lined with flat or cubical epithelium and contains deeply stained acidophilic colloid (fig. 2).

Changes associated with either type of goitre were not seen in the sheep, though goats and sheep slaughtered at Bareilly are reared in the same area. The explanation probably lies in the different feeding habits of these two species. Goats mainly live on tree leaves which may perhaps contain substances which inhibit the absorption of iodine or the leaves may contain less iodine than herbage does.

Summary

Thyroid glands were collected from goats and sheep at Bareilly slaughter house (U.P.-India). They were weighed and examined microscopically. About 10 per cent. of the goats were found to be goitrous. Goitre was more common in female and castrated male goats whilst not a single case of goitre was seen in sheep. The enlargement of the thyroid glands was found in goats and was not associated with other symptoms such as dwarfism.

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PLATE 1

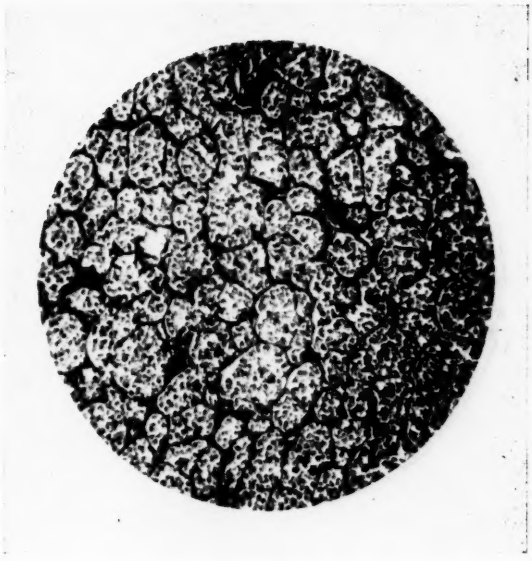

FIG. 1.

Thyroid in parenchymatous goitre in a goat showing proliferation of cells in to the lumen of the acini, absence of colloid and congested blood vessels.
Haematoxylin and Eosin X 100.

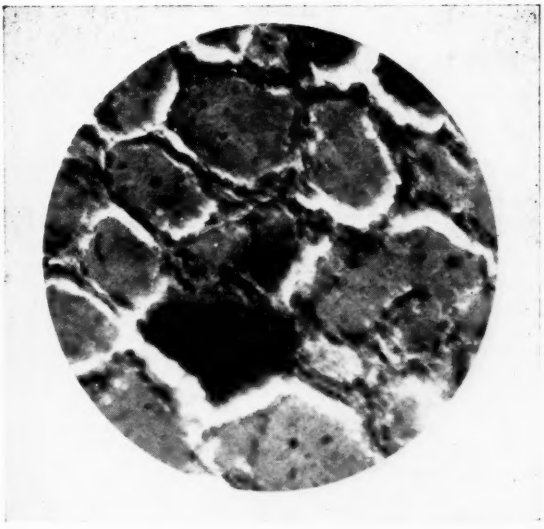

FIG. 2.

Thyroid from a normal goat showing acini lined with flat or cubical epithelium and containing colloid.
Haematoxylin and Eosin X 100

(Article by B. Dutt and N. D. Kehar on pages 176-178)

CORRESPONDENCE

1, Cedar Hill,
Newport, I.W.

The Editor,
The British Veterinary Journal. *March 25th,* 1959.

Dear Sir,

Your Editorial in the March issue, and the very successful operation on a donkey described therein, brings back memories of horse practice. As one interested in such procedures, and always willing to have a go, I congratulate the authors very warmly on the successful outcome of an historic operation.

While on this subject I would like to record two earlier operations, one an exploratory laparatomy on a foal, only two days old, for a suspended interssusception, which turned out to be an impaction, and had a successful outcome. The second was a "Caesarean" on a Clydesdale mare, which was a case of the operation being successful, but the patient died! In fact she appeared to be progressing well, but died very suddenly, apparently from shock the day following the operation, both these operations were performed during the summer of 1930, alas before we had the benefit of sulphonamide or penicillin.

Yours faithfully,

G. S. MUIR, D.V.M., M.R.C.V.S.

REVIEW

THE ÆTIOLOGY OF INFECTIOUS DISEASES WITH SPECIAL REFERENCE TO THE SUBSIDIARY AND IMPORTANT NON-SPECIFIC FACTORS. Lovell, Reginald (1959). 136 pp. 4 Figs. Michigan State University Press. 3.50 dollars.

During the spring of 1955 a series of lectures was given by the author in the Department of Microbiology and Public Health at Michigan State University. This small book contains the substance of the lectures.

The title suggests that the main attention is paid to those subsidiary factors which play their part in the causes of any disease. An infective agent may be one which habitually causes disease and may be classified as a pathogen. On the other hand the intrinsic differences between pathogens and non-pathogens may be trivial. Furthermore many of those agents classified as potential pathogens may exist in healthy animals and cause no harm. The state of the host and its relationship to the infective agent is thereby of great importance. The host-parasite relationship is an unstable one and this instability is associated with any change in environment and with the changing habits of man. Therefore, the natural history of the epidemic diseases is subject to great variation.

The first part of the book deals briefly with the attributes of bacteria which are classed as pathogens and with the normal bacterial flora of man and animals. The host is considered in the light of its normal protective mechanisms and of those stimulated by infection.

The second part of the book deals briefly with the subsidiary factors which play some part in infective diseases. Age, sex, trauma, stress, fatigue and changes of habits are discussed and illustrated with examples. It is pointed out that this book is intended to stimulate rather than to instruct and that the success or failure of disease control may depend upon an appreciation of subsidiary factors and the changing pattern of disease.

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NEWS

FIGHT AGAINST ANIMAL DISEASES IN HUNGARY

The number of veterinary surgeons in Hungary has risen in ten years from 1,100 to 1,700.

In an interview outlining the progress the country is making in enlarging and modernising its veterinary network, Mr. Oszkár Pusztai, Chief Director of the Veterinary Hygiene department of Hungary's Ministry of Agriculture, said "Ten years ago we had only one animal hygiene institute and one veterinary hospital in operation."

At the present time there are five animal hygiene institutes and 37 veterinary hospitals at work. In addition to these there are a new institute for vaccine production and 50 parasitological research stations.

A widespread publicity campaign using leaflets, pamphlets, lectures and film shows, has helped to spread information on animal hygiene among breeders and farmers.

The incidence of various contagious and other diseases has been reduced.

Speaking of future plans, Mr. Pusztai said the next aim was to rid the country's cattle of tuberculosis.

THE GREY MARES

The Vaux Breweries in Sunderland have imported seven French Percheron horses to improve the Company's stable of show horses.

The Percherons are the first to be imported into this country since the war.

When not competing or being exhibited they will earn their living by working within a six mile radius of Sunderland. Vaux teams have won the British Percheron Horse Society Challenge Cup for four-horse teams and the Pair Horse Trophy six times since 1953.

Percherons were first brought into this country after World War One. They are popular because of their docility and straight action.

The new horse will make up the Company's stud of draught horses to about thirty.

THE DAIRY INDUSTRY IN EL SALVADOR

Until a few years ago, milk production in El Salvador was of secondary economic importance to the growth of cotton, maize and sugar-cane. The cattle were reared for beef and were semi-wild: today about 200,000 of the 900,000 head of cattle in the country are dairy cows yielding about 2.4 litres per cow per day or some 480,000 litres for the population of 2,300,000.

Since 1952 F.A.O. and U.N.I.C.E.F. have helped towards this venture which depends on trained farm hands and dairy personnel. Calves have to be taught how to drink from pails; herdsmen have to be instructed how to teach them. Cows, which had never been milked without calves at their sides, had to be controlled and milked by herdsmen who had never milked a cow. The cows had to be dragged by lassos into stanchions. Schools for instruction in the dairy side of the cattle industry were established when the need for a balanced agricultural economy was apparent. Practical instruction is given in the management, handling, feeding and milking of dairy stock; pupils are taught about milk recording, natural and artificial insemination, the composition and preparation of concentrates, and the effect of different foods on milk, its quality and quantity. There are three courses of instruction which vary in length from one week to 3-4 weeks; the curriculum is basically the same but is presented according to the intellectual level of the participants and according to the employment of the student. Film shows and visits to places of interest make the courses attractive and the

prestige of students and school are enhanced by the presentation of certificates, at Agricultural Shows, to those students who have been successful in their examinations. The dairy farming industry of El Salvador is thus developing under the guidance of international organizations.

ANTIBIOTICS — A NEW BRITISH DISCOVERY

British scientists, engaged in antibiotic research, have made a major discovery. It opens the way to the evolution of many effective new penicillins. Four research workers at Beecham Research Laboratories, Brockham Park near Dorking, have isolated the basic molecule of penicillin. Professor E. B. Chain, the Nobel prizewinner, and an early worker on penicillin, has said that the discovery of the substance—6-Amino-Penicillanic Acid—could lead to the " most important breakthrough in the penicillin field since the discovery of penicillin's curative power ". Evidently Professor Chain's view was shared by other scientists in the United States. Beecham Research Laboratories have been approached by almost every large American pharmaceutical company engaged in antibiotic production and research with offers of collaboration in research and development.

Dr. J. Farquharson, Director of Research at Brockham Park, said that three urgent problems for which this new development offered promise of a solution were:

1. The widespread existence in hospitals of staphylococci resistant to the known penicillins.
2. Finding a weapon to combat gram negative organisms causing intestinal disorders, typhoid and other diseases, against which the known penicillins are almost valueless.
3. The development of new penicillins to existing ones.

Dr. Farquharson said " Because of the relative non-toxicity of the known penicillins, the field seems wide open to our researchers and in fact we have some very promising leads in these directions."

NOTICES

GLAXO LABORATORIES LTD, announce the introduction of the following new Canine Vaccines.

Dual Canilin containing one vial freeze-dried Canine Distemper Vaccine, one ampoule sterile distilled water and two ampoules Canine Virus Hepatitis Vaccine in a single patient pack.

Canilin-D containing one vial Canine Distemper Vaccine plus one ampoule sterile distilled water.

Canilin-H containing two ampoules of killed Canine Virus Hepatitis Vaccine—single patient pack.

Lepsolin containing 2 x 2 ml. ampoules of combined L. Canicola and L. icterohaemorrhagiae vaccine—single patient pack.

Also they announce the introduction of a mixed enterotoxaemia vaccine. This vaccine is for the protection of sheep against enterotoxaemia caused by Clostridium Welchii types C and D.

Price reductions for veterinary packs of Predsolan and Cytamen are being made from Monday, 6th April.

'DISTIVIT' B12 INJECTION VETERINARY — NEW PRICES

The Distillers Company (Biochemicals) Limited announces that the following new prices for the 'Distivit' B12 Veterinary brand of Injection of Vitamin B12 became effective from Monday, 6th April, 1959.

Pack	*Retail Price*
10 ml. vial (1,000 μg. per ml.)	24s. 0d.
30 ml. vial (250 μg. per ml.)	20s. 9d.

The prices quoted above are subject to usual discounts on orders received from the veterinary profession.

CHRISTY'S EMULSION

We have received a supply of Christy's Emulsion for the relief of minor Inflamatory Conditions of the Skin, such as rough and calloused hands, chapping, bruises and abrasions, and chafing. For Veterinary Surgeons who constantly have to have their hands in water and chemical solutions, etc., this preparation should prove a boon. When rubbed into the hands the emulsion leaves no trace of stickiness or greasiness, yet provides an effective shield against outside irritants. Christy's Emulsion may be obtained through your usual pharmaceutical agent.

Publisher's Notices

THE BRITISH VETERINARY JOURNAL, with which is incorporated THE VETERINARY JOURNAL, is published monthly, and copy for advertisements should be in the hands of the advertisement manager not later than the 20th of the preceding month, if proof is required. Tel.: Temple Bar 3386.

Letters for the Journal, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

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